OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.

Research Article
Differences in the Levels of Malondialdehyde, Total Cholesterol and Triglycerides after the Administration of a Passion Fruit Seed Ethanol Extract to Wistar Rats

[1]Dina Keumala Sari, [2]Marianne, [3]Sri Lestari and [4]Lidya Imelda Laksmi

[1]Department of Nutrition, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
[3]Department of Public Health, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia
[4]Department of Anatomy and Pathology, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia

Abstract

Background and Objectives: Passion fruit (*Passiflora edulis Sims*) seeds contain antioxidants and are expected to decrease the levels of cholesterol and malondialdehyde (MDA). The purpose of this study was to identify the effects of a passion fruit seed ethanol extract on Wistar rats fed atherogenic feed. **Materials and Methods:** The method of this study was a preclinical trial (post-test control group design) in rats by administering passion fruit seed ethanol extract for 14 days, using 26 male rats (*Rattus norvegicus*) aged two months, divided into 5 groups. The groups were a negative control group (atherogenic feed), a positive control group (standard feed) and test groups that were given the passion fruit seed ethanol extract at doses of 5 mg kg^{-1} body weight (BW), 10 mg kg^{-1} BW, or 20 mg kg^{-1} BW for 14 days to the Wistar rats fed with atherogenic feed. **Results:** The study results showed a significant difference in MDA levels, which was found in the group that was given passion fruit seed extract(10 mg kg^{-1} BW)and the positive control group that was given standard feed (mean±standard deviation: 1.83±0.40 mM vs 1.38±0.12 mM; p = 0.002). The level of total cholesterol also showed a significant difference, which was found in the group that was given passion fruit seed extract (10 mg kg^{-1} BW) with a negative control group that was given atherogenic feed (mean±standard deviation: 84.54±13.69 mg dL^{-1} vs 68.04±6.17 mg dL^{-1}; p = 0.003). **Conclusion:** Administration of passion fruit seed extract showed a significant difference in the level of triglycerides, which was found in the negative control group that was given atherogenic feed compared with the group that was given passion fruit seed extract at a dose of 5 mg kg^{-1} BW (mean±standard deviation: 1.09±0.30 mg dL^{-1} vs 0.77±0.25 mg dL^{-1}; p = 0.048).

Key words: Antioxidant, atherogenesis, atherogenic rats, flavonoid, free radicals, histopathology, lipid profile, *Passiflora edulis Sims*, piceatannol, purple passion fruit

Received: Accepted: Published:

Citation: Dina Keumala Sari, Marianne, Sri Lestari and Lidya Imelda Laksmi, 2019. Differences in the levels of malondialdehyde, total cholesterol and triglycerides after the administration of a passion fruit seed ethanol extract to wistar rats. Pak. J. Nutr., CC: CC-CC.

Corresponding Author: Dina Keumala Sari, Department of Nutrition, Faculty of Medicine, Universitas Sumatera Utara, Medan 20155, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A high incidence of atherosclerosis disease worldwide has caused numerous studies aiming to find more effective treatments and to prevent cardiovascular diseases. A high incidence of these diseases has also been seen in developing countries that have different dietary habits and lifestyles from those in developed countries; therefore, various types of therapy have been found, including the use of food products that are easily sourced with in the country or herbal products[1,2].

Theories explaining the cause of the atherosclerosis process have developed from high fat consumption, the inflammatory process and the oxidative stress theory. Oxidative stress occurs due to metabolic reactions that use excessive oxygen, resulting in the impairment of prooxidant and antioxidant homeostasis in cells. High fat consumption and excessive oxidative stress could cause increased lipid peroxidation processes and produce reactive aldehydes such as malondialdehyde (MDA)[3,4].

Plants containing phenolate compounds (bioflavonoids) could inhibit lipid peroxidation and have a strong antioxidant effect by cutting off the peroxy radical chain reaction through a scavenger effect. Bioflavonoid mixtures in food intake and supplementation have antioxidant and protective effects; one such food is passion fruit. Studies about passion fruits that contain bioflavonoids have developed with a focus on passion fruits but there are still a few studies addressing the passion fruit seeds[5-7].

Passiflora edulis Sims (*Passifloraceae*) is a woodbine frequently found in tropical countries. This plant is found in several colours, for example yellow, red and purple and fruit flesh is the form that is consumed most frequently[8,9]. There are many published studies about the fruit flesh and rinds of passion fruit but not about passion fruit seeds. The passion fruit is a typical plant that grows in the tropical region of North Sumatera, Indonesia. Passion fruit seed extract has been reported to contain polyphenol compounds that could show more effective antioxidant properties than the fruit flesh or rinds.

The polyphenols contained within passion fruit have shown an effect on body metabolism, such as lipid metabolism. The polyphenol compound contained in passion fruit is piceatannol, which has an antioxidant effect analogous to resveratrol[5]. Previous studies have shown that piceatannol in passion fruit has effects on insulin sensitivity improvement, improvement in lipid profiles, vasorelaxant effects, inflammation and oxidative stress reduction[5].

Passion fruits that grow in tropical regions, especially in North Sumatera, Indonesia, have different growing places with geographical environments and seasons that differ from other regions. With the variations among passion fruit, differences in the activity of antioxidants contained within the passion fruit are expected. The objective of this study was to identify the effect of the administration of a passion fruit seed ethanol extract on the lipid profile, malondialdehyde level and histopathological features of blood vessels in atherogenic Wistar rats for 14 days. The results of this study are expected to provide a profile of the passion fruit seed ethanol extract that could be used to decrease the lipid profile and malondialdehyde levels in Wistar rats. This product could increase the improvement of body metabolism and utilize passion fruit products in North Sumatera as local product utilization.

MATERIALS AND METHODS

This preclinical trial used an experimental trial design (post-test control group design). Study samples were 25 white *Rattus norvegicus* Wistar rats aged approximately 2 months and weighed 150-200 g. The rats were divided into 5 groups: treatment group with normal diet (C−), treatment group with atherogenic diet (C+) and treatment groups with atherogenic diet and administration of purple passion fruit seed ethanol extract at different doses (P2, P3 and P4). Purple passion fruit seed ethanol extract was given orally with an orogastric tube once daily for 14 days. The passion fruit seed ethanol extract was made previously at the Pharmacy Laboratory, Faculty of Pharmacy, University of North Sumatera, to be further given orally to the experimental rats.

Sample collection

Purple passion fruit: Purple passion fruits or *Passiflora edulis* in Latin have round egg shapes or are fully round and have diameters of approximately 4-6 cm. This variety of passion fruit is the most cultivated because of its most delicious taste and flavour. Usually, these types of passion fruits can be found in plateau areas with wet climates[5,10].

Passion fruits have a thin rind (0.5 mm) like a hard cork and they easily break when they are still raw and then they become flexible when they are ripe. In the fruit's cavity, there are dozens of black-coloured flat seeds that are approximately 0.5 cm with a very hard seed coat. The seed itself is in two pieces and is white-coloured. The seed coat is covered with thin pulp. This pulp is light yellow to orange-coloured. Fruit size, pulp thickness, flavour and acidity level have become the

standards to determine the quality of passion fruit. The larger the fruit size, the thicker the pulp with a higher level of flavour and acidity gives rise to a more qualified passion fruit. In Indonesia, there are 4 (four) types of cultivated passion fruits, which are purple passion fruit (*Passiflora edulis* var. edulis), *konyal* passion fruit (*Passiflora ligularis*), yellow passion fruit (*Passiflora edulis* var. flavicarpa) and erbis passion fruit (*Passiflora quadrangularis*)[11-14] (Fig. 1).

Purple passion fruit with Latin name (*Passiflora edulis* var. edulis) is sour purple passion fruit that has a round oval shape. The raw fruit is green-coloured but the ripe fruit is brown-purple, with fresh, sour taste and good flavour. Purple passion fruit is included in the *Passiflora edulis* species, *Passiflora* genus, *Malpighiales* order, *Spermatophyta* division and *Plantae* Kingdom, with binomial name of *Passiflora edulis*. This fruit takes 1.5 years to grow from seeding until the first harvest on a plateau. This plant is cultivated at an altitude of 1000 m above sea level but with certain treatments purple passion fruit can also be cultivated in middle ground or lowland, although this type is commonly processed into syrup or other processed products that have high economic value. The breeding method is the same as for the other type of purple fruit, which is by propagating it in trees or in loft or fence[9,15,16]. This study used passion fruit obtained from Berastagi Plantation, located 66 km south of North Sumatera's capital city, Medan City, 1300 m above sea level, with a latitude of 3.1853°N and a longitude of 98.5049°E.

Fig. 1: *Passiflora edulis Sims*

Fig. 2: The process of drying passion fruit seeds

Passion fruit seed ethanol extraction process: Passion fruit seed extract was made using a maceration method with 96% ethanol as the diluent and was conducted in Pharmacy Laboratory, Universitas Sumatera Utara, Indonesia. The sample used was the seeds of *Passiflora edulis* var *Sims*, weighing 10 kg (gross weight). Passion fruits were sliced in two to remove the seeds to be collected and cleaned from dirt (wet sorting), washed with running water until they were clean and then drained. The aim was to obtain passion fruit seeds that were free from fibre and fruit flesh. The seeds were then dried in open air protected from direct sunlight with continued drying using a drying cabinet (Indotrading, Indonesia) and then dried in an oven at a temperature of 40°C (Fig. 2). The dried seeds were then crushed using a blender (Miyako, Indonesia) until they became seed powder and sifted through20 mesh sieves.

Extract of passion fruit seed was made using the maceration method, which was performed using 96% ethanol, which had been previously distilled, with as much as 10 times the weight of passion fruit seed powder. A total of 1340 g of seed powder was put into container with 96% ethanol and the container was closed and left for three days protected from light with repeated stirring. After three days, the mixture was sifted and the leftover extract was dried. The result of the maceration was placed in a container and then distilled using a rotary evaporator device (Hei-VAP Rotary Evaporators, Heidolph, Germany) at a temperature of 45°C to separate the solution and steam so that an almost viscous extract was obtained. The almost viscous extract was then steamed in a waterbath (Griffin) until a viscous extract was obtained. The extract's net weight was then determined and the extraction process resulted in a yield of 134.15 g.

Preparation of the passion fruit seed sample: Preparation of passion fruit seed ethanol extract at the dose of 5 mg kg^{-1} body weight for the rats was conducted by weighing 5 mg of passion fruit seed ethanol extract. The extract was then put into a mortar and slowly crushed. A solution of CMC Na (0.5%) was added slowly and crushed until homogenous. The suspension was then placed into a 10 mL volumetric flask and CMC Na solution was added to the limit mark. The same

method was applied to the creation of passion fruit seed ethanol extract doses of 10 mg kg^{-1} BW and 20 mg kg^{-1} BW. The extract was given to the rats by an orogastric tube once daily by creating a suspension of the passion fruit seed ethanol extract using a 0.5% solution of carboxymethyl cellulose natrium (CMC Na).

Experimental animals

Ethical approval: This study was approved by the ethical principle in an experimental trial study with experimental animals by The Animal Research in Biology Faculty of Universitas Sumatera Utara, with ethical number 112/KEPH-FMIPA/2018. In total, 25 young Wistar rats (*Rattus norvegicus*) weighing 150-200 g were obtained.

Conditioning of experimental animals: All experimental rats were acclimated in the experimental cages for two weeks to standardize the way of living, eating and condition of the experimental cage. Rats were placed in an experimental cage at room temperature with 12 h of exposure to bright light and darkness alternately. All rats were fed commercial standard feed and water *ad libitum*. Bedding for rats came from sterilized coarse sawdust and was changed twice a week. Lighting used was natural light (from a window) with room temperature (normal). The cage was also equipped with an exhaust fan to maintain air flow and to remove excess heat.

Grouping of experimental animals: This experimental trial was conducted in The Animal Research Laboratory in Biology Faculty of Universitas Sumatera Utara, Medan, North Sumatera, Indonesia and all experimental rats were divided into five groups, which were the negative control (C–) consisting of 6 rats, the positive control (C+), treatment 1 (P1), treatment 2 (P2) and treatment 3 (P3), which each consisted of 5 rats. The C– group was given 1 additional rat to anticipate the death of the rats due to atherogenic feed. Rats in the C+ group were only given standard feed and rats in the C– group were only given atherogenic feed. Grouping for the C groups was based on the hypothesis that the administration of atherogenic feed in the C– negative group would result in a negative effect, whereas the group that was given standard feed would result in a positive effect. Moreover, the treatment groups were divided into three groups, which were 1, 2 and 3 and each were given atherogenic feed and one quail egg yolk daily for 14 days, which was conducted to induce atherogenic rats.

After the induction time, the control group was still given the standard feed, whereas group 1 was given only atherogenic feed, group 2 was given atherogenic feed and passion fruit seed ethanol extract at a dose of 5 mg kg^{-1} body weight, group 3 was given atherogenic feed and passion fruit seed ethanol extract at a dose of10 mg kg^{-1} body weight and group 4 was given atherogenic feed and passion fruit seed ethanol extract at a dose of 20 mg kg^{-1} body weight. Administration of passion fruit seed ethanol extract was conducted for 14 consecutive days nonstop. The rats body weights were measured at the beginning of the study and during blood sampling.

Experimental animal data collection: The collected data consisted of rat body weights obtained by weighing rats using a weight scale (Sartorius Melter brand) with an accuracy of 0.1 kg once a week. Daily feed intake data were measured by weighing the leftover feed given to experimental animals every day using a weight scale (Sartorius Melter brand) with an accuracy of 0.1 kg.

After 14 days of treatment, the rats were fasted for 10 h and then blood sampling was performed by cardiac puncture. Before blood sampling, the rats were anaesthetized with an ether solution to be further euthanized. Blood was withdrawn (as much as 3 mL) and centrifuged at 3500 rpm for 5 min. Laboratory tests of total cholesterol, LDL, HDL, triglyceride and MDA levels were further conducted by taking blood samples. The lipid profile and MDA levels were checked using a spectrophotometry monochromator method using a spectrophotometer device (Thermo Scientific™ Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific Laboratory Equipment (LPG), United States).

Additionally, examination of blood vessels was performed by the histopathological examination of the formation of sponge cells. This examination was performed by taking the aortic arch to conduct histopathological examination of the formation of sponge cells. The tissue was stained using Oil RedO (OR-O) and haematoxylin eosin (HE).

Experimental animals feed intake: The standard feed contained isocaloric standard food, whereas the atherogenic feed contained a high level of fat. Additionally, one quail egg yolk was given daily to increase the cholesterol levels in each rat's blood (Table 1). Egg yolk could increase the level of lipids

Table 1: Composition of rat's food

Ingredients	Standard Intake	Atherogenic Intake
Confeed PAR-S (g)	200	200
Wheat (g)	100	100
Glucose (g)	-	80
Cholesterol (g)	-	8
Cholic acid (g)	-	0.8
Coconut oil (g)	-	40
Water (mL)	71.2	71.2

Table 2: Phytochemical screening analysis

Sample	Identity and sample	Parameter		Results	Unit	Technique of analysis
Passiflora edulis Sims. seed ethanol extract		Phytochemical:				
		Flavonoid		Positive	-	
		Alkaloid	Wagner	Negative	-	
			Mayer	Negative	-	
			Dragendrof	Negative	-	
		Tannin		Positive	-	Colour visualization
	Solids	Saponin		Positive	-	
		Quinone		Negative	-	
		Steroid		Negative	-	
		Triterpenoid		Positive	-	

in the blood, so hyperlipidaemia in experimental animals could be achieved. It is known that cholesterol from egg yolk is a lipid component consisting of 65.5% triglycerides, 5.2% cholesterol and 28.3% phospholipids. Additional egg yolk was given during the acclimatization period and treatment period, which was 1 egg yolk/rat/day given *ad libitum* in the morning and evening.

Content analysis

Phytochemical screening and antioxidant test (DPPH method): Phytochemical screening was conducted by performing a test, whereas the antioxidant test was performed according to the method of the phytochemical analysis and antioxidant test IC_{50}-DPPH, conducted in the Central Laboratory of Biopharmaceutical Study, Research and Community Service Body, Bogor Agricultural Institute, Indonesia, with certificate number 405.013/LPSB/IPB/V/2018.

Statistical analysis: Data obtained from the research results were analysed and processed using SPSS 20. Data analysis was started by a data normality test using the Shapiro-Wilk test. To identify differences from each treatment, one-way ANOVA was performed, followed by the least significant difference (LSD) test. For abnormal distributed data, the Kruskal-Wallis test was performed, followed by a double comparison, which was the Mann-Whitney test. Significance was determined with a $p<0.05$.

RESULTS

Phytochemical screening and antioxidant analysis: The flavonoid test results showed the presence of flavonoids in the passion fruit seed extract, along with tannin, saponin and triterpenoid substances, as presented in Table 2. Furthermore, the antioxidant activity of passion fruit seed extract was tested using the DPPH radical (2,2-diphenyl-1-picrylhydrazyl), by which the antioxidant compounds will react with DPPH radicals. The antioxidant mechanism of action is the donation

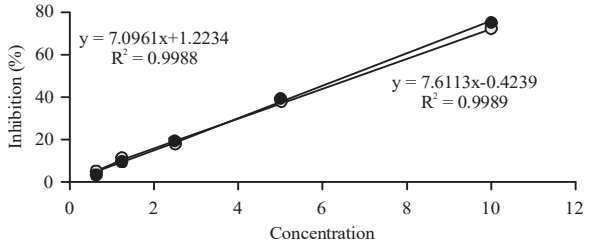

Fig. 3: Comparison between the concentration and inhibition of free radical absorbance

of hydrogen atoms, which will cause DPPH discoloration from purple to yellow measured at a wavelength of 517 nm[17]. The calculated parameter from this DPPH method is the 50% inhibitory concentration (IC_{50}), or the concentration that can reduce free radical activity by 50%.

The passion fruit extract was made into several concentrations and tested using DPPH radicals. The purpose of making some of these concentrations was to find IC_{50} values using mathematical equations obtained through the correlation between the inhibition and extract concentration. Inhibition is the presentation of purple discoloration and can be calculated from its absorbance. At each extract concentration, free radicals will be given and allowed to react for 30 min, with the effective time for reaction of the test sample and DPPH being 30 min due to entry into the propagation stage. The relationship between the concentration and inhibitory percentage of passion fruit seed extract using ethanol as the solvent is shown in Fig. 3.

The results obtained by the extract concentration value are directly proportional to the inhibitory value. The higher the concentration, the higher the inhibitory value. Fig. 3 also shows that the greater the concentration, the more antioxidant content in the extract, which can reduce free radical activity (marked by the discoloration of purple colour from DPPH). Antioxidant testing was also carried out on vitamin C (ascorbic acid) as a positive control and for comparison. A control is intended to test the validity of a

Table 3: Analysis of antioxidants using the DPPH method

Sample	Sample condition	Parameter	Result	Unit	Technique of analysis
Ethanol	Solid	Antioxidant IC_{50}-DPPH	<31.25	ppm	Spectrophotometry
Standard vitamin C	Solid	Antioxidant IC_{50}-DPPH	6.75	ppm	Spectrophotometry

Table 4: Mean values of body weight at the beginning and end of the study

Groups	n	Body weight at the beginning (g)	Body weight at the end (g)	p-value
C−	6	189.40±16.18	221.20±19.25	0.006*
C+	5	167.00±5.74	185.20±6.45	0.050
P1	5	199.20±15.05	230.60±16.01	0.014*
P2	5	196.17±13.12	238.50±33.6	0.001*
P3	5	196.20±7.66	216.00±17.07	0.032*

*Significant difference, Paired t-test, C−: Negative control group, C+: Positive control group, P1: 5 mg kg^{-1} treatment group, P2: 10 mg kg^{-1} treatment group, P3: 20 kg kg^{-1} treatment group

method (comparing the results of research with other studies that have been done). The correlation between concentration and inhibition in vitamin C is illustrated in Fig. 3 and has a high correlation coefficient of 0.998.

The effectiveness of a sample to counteract free radicals from the DPPH method is named the IC_{50}. The definition of IC_{50} is the concentration that can reduce 50% of the DPPH free radicals. The smaller the IC_{50} value, the greater the antioxidant activity. IC_{50} values from passion fruit seed extract using ethanol as the solvent and vitamin C are presented in Table 3.

A compound is said to have a very strong antioxidant activity if the IC_{50} value is less than 50 ppm, the strong group has an IC_{50} between 50-100 ppm, the medium group has an IC_{50} value between101-150 ppm and the weak group has an IC_{50} value between150-200 ppm[17]. Based on the previous statement, it can be said that passion fruit seed extract using ethanol as the solvent and vitamin C both have very strong antioxidant activity and the ethanol extract is equivalent to vitamin C in counteracting free radicals.

Weight: At the beginning of the study, the rat body weights in each group showed significant differences between the groups. The mean body weights of all rats before treatment was 189.8±16.34 g and after treatment this increased to 219.08±26.99 g, with significant differences for each group for body weight before and after treatment in each group ($p = 0.003$ and $p = 0.006$, respectively).

The results of this study showed the percent of feed intake of the rats fed standard food, atherogenic food, atherogenic food added with passion fruit extract of 5 mg kg^{-1} body weight, atherogenic food added with passion fruit extract of 10 mg kg^{-1} body weight and atherogenic food added with passion fruit extract of 20 mg kg^{-1} body weight had increased body weights in each group. In this study, the weight of all rats before the study showed differences between the groups with a mean body weight of 189.85±16.34 g. After the treatment period, there was an increase in rat body weight to 219.08±26.99 g ($p = 0.001$), with an average difference in body weight before and after the treatment of 29.23±19.89 g.

The body weights of the rats were measured every day to monitor the progress of the rat's body weight. Table 4 shows the difference in body weight between the beginning and the end of the study to determine the relationship between the changes in body weight and rat food intake. The results showed a significant difference ($p<0.05$) between the initial and final body weights of the study in the four groups. There was no significant increase in the C+ group, namely, the group that was given standard food. Each group experienced an increase in body weight, which was most apparent in the P2 group. After being tested by statistics, the most significant difference was observed in the C− and P2 groups ($p = 0.001$).

This difference in body weight is influenced by the intake of atherogenic food, which is higher in fat composition than standard food. The increase in fat levels affects changes in body weight, lipid profiles and levels of MDA. Fat composition in atherogenic food intake is greater, which is equal to 47.3% of total energy compared to standard food intake, of which fat is only 8% of the total energy. The addition of quail egg yolks for 14 days continuously gave additional cholesterol to the intake of the group of rats with atherogenic food, which caused dyslipidaemia and triggered the acceleration of the atherogenic process in experimental rats.

The administration of passion fruit seed extract showed no significant difference. The treatment group and the atherogenic food-fed group all showed significant differences in weight; only the group that was given standard food had an increase but did not show significant differences before and after treatment.

Effects on MDA and lipid profiles: Wistar rats were given treatment for 14 days and on the last day of the study, rats were fasted for 12 h by taking out all food and drinks from their cages, then taking blood and evaluating them according

Table 5: Mean values of total cholesterol level before and after treatment

Group/parameters	C-	C+	P1	P2	P3	p-value
Triglycerides (mg dL^{-1})	1.09±0.30	0.75± 0.17	0.77±0.25	0.74±0.23	0.48±0.25	0.014*
Total cholesterol (mg dL^{-1})	84.54±13.69	73.39±5.5	74.51±4.31	68.04±6.17	68.83±8.42	0.029*
HDL (mg dL^{-1})	60.58±13.47	52.50±10.85	53.91±8.04	48.28±4.78	45.32±12.64	0.199
LDL (mg dL^{-1})	21.17±4.31	23.62±5.79	21.05±3.45	20.36±2.14	18.83±4.72	0.502
MDA (mM)	1.62±0.07	1.83±0.41	1.59±0.17	1.38±0.12	1.44±0.12	0.021*

*Significant difference, ANOVA test, C-: Negative control group, C+: Positive control group, P1: 5 mg kg^{-1} treatment group, P2: 10 mg kg^{-1} treatment group, P3: 20 kg kg^{-1} treatment group

to the research protocol. The results obtained showed that the there were differences in MDA levels in the Wistar rats serum among the various groups (ANOVA test, p = 0.021). The post hoc Tukey test showed that there were two doses of passion fruit seeds that showed significant differences. This difference was seen between the group given standard food and the P2 group. This indicates that the passion fruit extract given shows a significant difference compared with standard food (C+) but not with atherogenic food (C-). The administration of passion fruit seed extract at the dose of 20 mg kg^{-1} body weight (P3) showed a significant difference from the standard food group. This illustrates that the administration of passion fruit seed extract of 10 mg kg^{-1} for 14 days decreases oxidative stress compared to the standard group. This significant difference illustrates the low emphasis on MDA levels in the 10 mg kg^{-1} passion fruit seed extract dose group accompanied by the atherogenic diet compared with standard food (mean MDA levels 1.38±0.12 vs 1.83±0.4 µM, respectively). However, a significant difference was not found between each group that was given atherogenic food. Based on the results obtained, passion fruit seed extract was not able to show a significant difference in decline in the C- and P1 groups, although the lowest level of MDA was found in the P2 group. In this case, administration of passion fruit seed extract at a dose of 10 mg kg^{-1} showed significant differences compared to the other doses of the extract (Table 5).

The results of this study indicate that atherogenic food given to rats for 14 days gave an increase in all lipoproteins, including triglycerides, total cholesterol and HDL but not LDL. Atherogenic food contains a high fat content ranging from 25-35% of the total energy. A high fat intake for 14 days affected lipoprotein levels and a significant difference was observed in the total cholesterol and triglycerides, whereas for HDL, only a significant difference was seen between the C+ and C- groups (52.51±10.85 mg dL^{-1} vs 60.59±13.47 mg dL^{-1}). Among the treatment groups, administration of passion fruit seed extract showed the highest mean value in group P1, namely, 5 mg kg^{-1} body weight (Table 5).

An examination of LDL levels also showed no significant difference between all groups; the highest increase was observed in the group with standard feed (C+). Interestingly, the lowest LDL level was observed in the treatment groups, especially in the P3 group, which was given 20 mg kg^{-1} body weight of the extract. The treatment groups P1 and P2 showed a decrease in LDL levels but did not show significant differences compared with the C- group. The duration of treatment might be a possible factor for the fact that no differences were shown in each group. In addition, although all the treatment groups were given passion fruit seed extract, atherogenic food was still given and with this treatment design, low LDL levels can be seen in all treatment groups (Table 5).

The groups that showed the significant differences in total cholesterol levels was the P2 and P3 with the administration of passion fruit seed extract of 5 and 10 mg kg^{-1} body weight compared with the C- group (atherogenic food feeding). The lowest average cholesterol level was found in the P2 group. The highest total cholesterol level was found in the C- group. The administration of passion fruit seed extract showed a decrease in the mean total cholesterol level in the groups according to the number of doses given. The larger the dose, the lower the average total cholesterol level obtained and the minimum value found in the P3 group was 56.56 mg dL^{-1} (Table 5). Passion fruit seed extract containing antioxidants can inhibit the rate of increase in total cholesterol and triglyceride levels, especially in rats given atherogenic food for14 days. Flavonoids contained in passion fruit seed extract can inhibit an increase in total cholesterol levels by a mechanism that inhibits the activity of the enzyme HMG CoA reductase, which plays an important role in cholesterol biosynthesis.

Based on the LSD test, there were significant differences in each parameter but the treatment group that showed the most significant difference indicating the effect of passion fruit seed extract over14 days with atherogenic food was the P2 group (10 mg kg^{-1} body weight dose of passion fruit seed extract). The most significant results were shown the P3 group (dose of passion fruit extract 20 mg kg^{-1} body weight) with atherogenic food with respect to the triglyceride levels (Table 6).

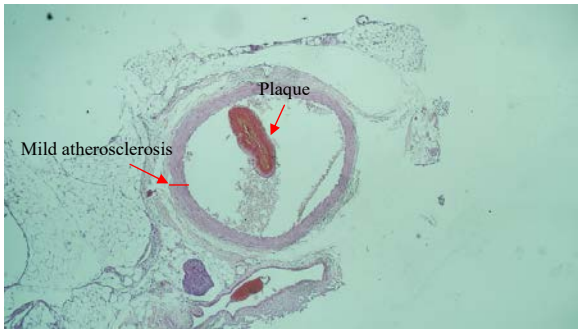

Fig. 4: Plaque and atherosclerosis in C– rat aortas

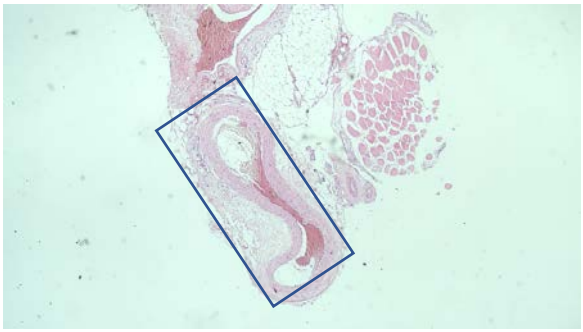

Fig. 5: Normal artery wall in the P2 group, filled with erythrocyte

Table 6: Significant changes in triglycerides, total cholesterol and MDA levels

Parameters	Different groups	p-value
Triglycerides	C+ and C–	0.036
	C– and P1	0.048
	C– and P2	0.025
	C– and P3	0.001
Total cholesterol	C– and C+	0.044
	C– and P2	0.003
	C– and P3	0.007
MDA	C+ and P2	0.002
	C+ and P3	0.009

Blood vessel histopathology: At the end of treatment, (on the 15th day), the rats were euthanized, the aortic arc was taken and histopathological examination was performed to see the formation of plaques, which would describe the process of atherosclerosis. Tissue was stained using Oil RedO (OR-O) and haematoxylin eosin (HE) stains.

Histopathological examination of the aortic arc showed that in the C– group, plaques and thickening of the aortic arc wall were formed, which described the process of atherosclerosis (Fig. 4). In group P3, atherosclerosis was seen in part of the arterial wall but congestion was seen on the lumen, where erythrocyte cell accumulation occurred and no plaque was found inside (Fig. 5).

DISCUSSION

Passion fruit is a fresh fruit in the Asian region, especially Indonesia (North Sumatra), which can be used as a drug because it contains high levels of flavonoids, tannins and antioxidants.[5] Seeds, leaves and mesocarp of passion fruit can be used in the pharmaceutical industry but in North Sumatra, purple passion fruit (*Passiflora edulis*) is generally only used as a raw material for making syrup. This new study shows the presence of phenol levels in passion fruit seed extracts, although not yet measuring the phenol content.

Previous research stated that passion fruit rinds showed higher antioxidant activity than passion fruit pulp and was especially effective on the production of reactive oxygen species (ROS) and myeloperoxidase (MPO) activity, which play a role in the inflammatory process[18]. That study did not examine passion fruit seeds and the pulp was made by separating the porridge from the seeds[8,18]. The active ingredient in passion fruit seeds is piceatannol in all types of passion fruit variants, including yellow passion fruit[19,20].

The ability of piceatannol to inhibit linoleic acid peroxidation is better than other antioxidants, including beta carotene and piceatannol shows a variety of biological activities, including antioxidant, anticarcinogenic, antiatherogenic and anti-inflammatory activities. Phenol compounds inhibit lipid peroxidation and the lipo oxygenation process by terminating peroxyl radical chain reactions through cleaning effects (scavenger), similar to vitamin C, beta carotene and vitamin E[19].

The results of this study indicate that antioxidant activity correlates with flavonoid levels. DPPH capture activity by passion fruit seed extract showed a very strong relationship between the content of polyphenols and free radical capture activity. The antioxidant components of phenolic compounds work by cleaning reactive oxygen species (ROS), with inhibition that is better than other antioxidants, such as beta carotene[19,21].

Previous research has shown that the bioavailability of passion fruit seeds is influenced by the content of the matrix contained in them and the form of the passion fruit seed extract is better than other forms. It is important to emphasize that the food matrix is complex and that there are synergistic or antagonistic interactions of each compound[19,21,22]. Other antioxidants or vitamins within the food matrix also influence each other but in this study, the food matrix was not shown to contain other antioxidants or vitamins.

Lipid peroxide level measurement is used as an indicator of cell and tissue oxidative stress. Lipid peroxides are unstable and decompose, resulting in a number of compounds,

including reactive carbonic compounds. Polyunsaturated fatty acids decompose to produce malondialdehyde (MDA)[23,24]. The results of this study indicate a difference in MDA levels between the groups of rats, especially after the administration of passion fruit seeds. This difference illustrates the inhibition of fat peroxidation by giving passion fruit seed extract. The role of piceatannol in the passion fruit seed extract acts similarly to resveratrol as an antioxidant[5], the antioxidant's working power is strong and the possible pathway is to terminate the peroxyl radical chain reaction through a cleansing effect on ROS such as OH·, ONOOH· and HOCl·[21,25,26].

This study showed a significant difference in MDA levels with a dose of 10 mg kg^{-1} body weight within 14 days in rats given atherogenic food compared to standard food. This will provide an overview for human studies taking into account the dosage and duration of administration. MDA levels can be used as a marker of oxidative stress in various inflammatory reactions in the body, both in total or free form MDA.[27] Pathological changes caused by various types of diseases indicate endogenous and exogenous MDA in living cells. There is an increase in MDA levels due to stimulation by H_2O_2 in a high oxidative pressure environment[28]. Differences in MDA levels in experimental rats illustrate that there is a decrease in oxidative stress levels.

This study showed a difference in total cholesterol and triglyceride levels. Differences in total cholesterol levels after supplementation of passion fruit seeds to experimental rats can be reflected in the metabolism of the human body. The human body, with a lifestyle of a high intake of saturated fat, trans fat and cholesterol, causes fat build-up and an inflammatory reaction that triggers the process of atherogenesis, which will eventually lead to atherosclerosis[29-31].

Differences in cholesterol levels at a dose of 20 mg kg^{-1} body weight after14 days can make the active ingredient in passion fruit seed extract used to reduce the total cholesterol levels in the body. High cholesterol concentrations, especially LDL levels, are expressed as a cause of atherosclerosis. Atherosclerotic lesions begin with the oxidation of LDL, which causes the endothelium to express monocyte attachment, produce monocytic chemotactic proteins and stimulation factors for macrophage colonies and to attach to blood vessel walls to form foam cells, ending with the formation of initial lesions known as plaques[30,32]. In this study, the presence of plaques in the histopathological picture and the process of blood vessels that formed atherosclerosis were seen but after the administration of passion fruit seed extract, minimal plaque formation and no blood vessel formation of atherogenesis were seen.

In previous studies conducted on human subjects, passion fruit seed extract was administered to non-overweight and overweight men and women without other metabolic disorders. The role of piceatannol contained within the passion fruit seeds was observed on insulin levels, HOMA-IR, blood pressure and heart rate; the results showed a decrease in these parameters, especially in male subjects who were overweight[5]. The results of the study did not show any effect on the markers of oxidative stress and lipid profiles, probably due to the small number of samples, the number of short administrations (8 weeks) and the absence of effective doses. Several previous studies have examined the role of nutrition in the prevention of cardiovascular disease. Research in people who consume red wine found that these people rarely suffer heart attacks compared to people who do not consume red wine. It has been suggested that red wine contains resveratrol, which has a cardio-protective effect and piceatannol is stated to have a similar working power toresveratrol[5,33,34]. The limitation of this study was the short duration of the trial period, so the study was not able to show a difference in the levels of LDL or the antioxidant or vitamin content in the passion fruit seed extract. This study also did not mention the level of piceatannol. Further study in humans is needed to test the effectiveness of this herbal product.

CONCLUSION

Passion fruit extract (*Passiflora edulis Sims*) has a potential effect to reduce MDA, total cholesterol and triglyceride levels in rats. The passion fruit seed extract can be used as an alternative to hold back the rate of increase in total cholesterol and MDA levels with atherogenic intake. With further research, it is hoped that the role of passion fruit seeds will be further known when low fat and low cholesterol foods are given, so that this extract can be applied to prevent the process of atherosclerosis.

ACKNOWLEDGMENTS

The authors would like acknowledge one of the researchers, Sri Lestari, (who died when this research was in its final stages) for assistance with statistical analysis. The authors would also like to thank the Ministry of Research and Technology and Higher Education, Republic of Indonesia for funding this research. The support is under the research of the USU of Year TALENTA grant 2018 Contract Number 2590/UN5.1.R/PPM/2018.

REFERENCES

1. Dziedzic, E.A., J.S. Gąsior, M. Pawłowski, B. Wodejko-Kucharska, T. Saniewski, A. Marcisz and M.J. Dąbrowski, 2019. Vitamin D level is associated with severity of coronary artery atherosclerosis and incidence of acute coronary syndromes in non-diabetic cardiac patients. Arch. Med. Sci., 15: 359-368.
2. Duś-Żuchowska, M., J. Bajerska, P. Krzyżanowska, A. Chmurzyńska, A. Miśkiewicz-Chotnicka, A. Muzsik and J. Walkowiak, 2018. The central European diet as an alternative to the mediterranean diet in atherosclerosis prevention in postmenopausal obese women with a high risk of metabolic syndrome - a randomized nutrition-al trial. Acta Scientiarum Polonorum Technol. Aliment., 17: 399-407.
3. Savoca, M.R., L.M. Steffen, A.G. Bertoni and L.E. Wagenknecht, 2017. From neighborhood to genome: Three decades of nutrition-related research from the atherosclerosis risk in communities study. J. Acad. Nutr. Diet., 117: 1881-1886.
4. Steinberger, J., S.R. Daniels, R.H. Eckel, L. Hayman, R.H. Lustig, B. McCrindle and M.L. Mietus-Snyder, 2009. Progress and challenges in metabolic syndrome in children and adolescents a scientific statement from the American Heart Association atherosclerosis, hypertension and obesity in the young committee of the council on cardiovascular disease in the young: Council on cardiovascular nursing; and council on nutrition, physical activity and metabolism. Circulation, 119: 628-647.
5. Kitada, M., Y. Ogura, H. Maruki-Uchida, M. Sai and T. Suzuki *et al*., 2017. The effect of piceatannol from passion fruit (*Passiflora edulis*) seeds on metabolic health in humans. Nutrients, 10.3390/nu9101142
6. Kandandapani, S., A.K. Balaraman and H.N. Ahamed, 2015. Extracts of passion fruit peel and seed of *Passiflora edulis* (Passifloraceae) attenuate oxidative stress in diabetic rats. Chin. J. Nat. Med., 13: 680-686.
7. Maruki-Uchida, H., I. Kurita, K. Sugiyama, M. Sai, K. Maeda and T. Ito, 2013. The protective effects of piceatannol from passion fruit (*Passiflora edulis*) seeds in UVB-irradiated keratinocytes. Biol. Pharm. Bull., 36: 845-849.
8. Zeraik, M.L., D. Serteyn, G. Deby-Dupont, J.N. Wauters and M. Tits *et al*., 2011. Evaluation of the antioxidant activity of passion fruit (*Passiflora edulis* and *Passiflora alata*) extracts on stimulated neutrophils and myeloperoxidase activity assays. Food Chem., 128: 259-265.
9. Wong, Y.S., C.M. Sia, H.E. Khoo, Y.K. Ang, S.K. Chang and H.S. Yim, 2014. Influence of extraction conditions on antioxidant properties of passion fruit (*Passiflora edulis*) peel. Acta Scientiarum Polonorum Technol. Aliment., 13: 257-265.
10. Silva, S.R., N.M. Almeida, K.M.M. de Siqueira, J.T. Souza and C.C. Castro, 2019. Isolation from natural habitat reduces yield and quality of passion fruit. Plant Biol., 21: 142-149.
11. Nerdy, N. and K. Ritarwan, 2019. Hepatoprotective activity and nephroprotective activity of peel extract from three varieties of the passion fruit (*Passiflora* sp.) in the albino rat. Open Access Maced. J. Med. Sci., 7: 536-542.
12. Albuquerque, M.A.C., D.S. Yamacita, R. Bedani, J.G. LeBlanc and S.M.I. Saad, 2019. Influence of passion fruit by-product and fructooligosaccharides on the viability of *Streptococcus thermophilus* TH-4 and *Lactobacillus rhamnosus* LGG in folate bio-enriched fermented soy products and their effect on probiotic survival and folate bio-accessibility under *in vitro* simulated gastrointestinal conditions. Int. J. Food Microbiol., 292: 126-136.
13. Albuquerque, M.A.C., R. Bedani, J.G. LeBlanc and S.M.I. Saad, 2017. Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. Int. J. Food Microbiol., 261: 35-41.
14. Alamar, P.D., E.T.S. Caramês, R.J. Poppi and J.A.L. Pallone, 2016. Quality evaluation of frozen guava and yellow passion fruit pulps by NIR spectroscopy and chemometrics. Food Res. Int., 85: 209-214.
15. Matsui, Y., K. Sugiyama, M. Kamei, T. Takahashi, T. Suzuki, Y. Katagata and T. Ito, 2010. Extract of passion fruit (*Passiflora edulis*) seed containing high amounts of piceatannol inhibits melanogenesis and promotes collagen synthesis. J. Agric. Food Chem., 58: 11112-11118.
16. Jardim, B.C., V.A. Perdizio, M.A. Berbert-Molina, D.C. Rodrigues and S. Botelho-Junior *et al*., 2010. Herbivore response in passion fruit (Passiflora edulis Sims) plants: induction of lipoxygenase activity in leaf tissue in response to generalist and specialist insect attack. Protein Pept. Lett., 17: 480-484.
17. Molyneux, S.L., C.E. Lister and G.P. Savage, 2004. An investigation of the antioxidant properties and colour of glasshouse grown tomatoes. Int. J. Food. Sci. Nutr., 55: 537-545.
18. Simirgiotis, M.J., G. Schmeda-Hirschmann, J. Bórquez and E.J. Kennelly, 2013. The *Passiflora tripartita* (Banana passion) fruit: a source of bioactive flavonoid C-Glycosides isolated by HSCCC and characterized by HPLC–DAD–ESI/MS/MS. Molecules, 18: 1672-1692.
19. De Santana, F.C., L.R. de Oliveira Torres, F.B. Shinagawa, A.M. de Oliveira e Silva and L.T. Yoshime, *et al*., 2017. Optimization of the antioxidant polyphenolic compounds extraction of yellow passion fruit seeds (*Passiflora edulis* Sims) by response surface methodology. J. Food Sci. Technol., 54: 3552-3561.
20. De Souza, C.G., T.H. Rodrigues, L.M. e Silva, P.R. Ribeiro and E.S. de Brito, 2018. Sequential extraction of flavonoids and pectin from yellow passion fruit rind using pressurized solvent or ultrasound. J. Sci. Food Agric., 98: 1362-1368.

21. Dos Reis, L.C.R., E.M.P. Facco, S.H. Flôres and A. de Oliveira Rios, 2018. Stability of functional compounds and antioxidant activity of fresh and pasteurized orange passion fruit (*Passiflora caerulea*) during cold storage. Food Res. Int., 106: 481-486.
22. Dos Reis, L.C.R., E.M.P. Facco, M. Salvador, S.H. Flôres and A. de Oliveira Rios, 2018. Antioxidant potential and physicochemical characterization of yellow, purple and orange passion fruit. J. Food Sci. Technol., 55: 2679-2691.
23. Ahmad, R., A.K. Tripathi, P. Tripathi, S. Singh, R. Singh and R.K. Singh, 2008. Malondialdehyde and protein carbonyl as biomarkers for oxidative stress and disease progression in patients with chronic myeloid leukemia. *In vivo*, 22: 525-528.
24. Chilakapati, S.R., M. Serasanambati, P.K. Manikonda, D.R. Chilakapati and R.R. Watson, 2014. Passion fruit peel extract attenuates bleomycin-induced pulmonary fibrosis in mice. Can. J. Physiol. Pharmacol., 92: 631-639.
25. Gerritsen, W.B., W.J.P. van Boven, D.S. Boss, F.J. Haas, E.P. van Dongen and L.P. Aarts, 2006. Malondialdehyde in plasma, a biomarker of global oxidative stress during mini-CABG compared to on- and off-pump CABG surgery: A pilot study. Interact. Cardiovasc. Thoracic Surg., 5: 27-31.
26. Jayahari, N.K., N.T. Niranjan and A. Kanaparthy, 2014. The efficacy of passion fruit juice as an endodontic irrigant compared with sodium hypochlorite solution: An *in vitro* study. J. Invest. Clin. Dent., 5: 154-160.
27. Cui, X., J. Gong, H. Han, L. He and Y. Teng *et al*., 2018. Relationship between free and total malondialdehyde, a well-established marker of oxidative stress, in various types of human biospecimens. J. Thoracic Dis., 10: 3088-3097.
28. Chen, J., L. Zeng, T. Xia, S. Li and T. Yan *et al*., 2015. Toward a biomarker of oxidative stress: A fluorescent probe for exogenous and endogenous malondialdehyde in living cells. Anal. Chem., 87: 8052-8056.
29. Raggi, P., J. Genest, J.T. Giles, K.J. Rayner, G. Dwivedi, R.S. Beanlands and M. Gupta, 2018. Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. Atherosclerosis, 276: 98-108.
30. Torres, N., M. Guevara-Cruz, L.A. Velázquez-Villegas and A.R. Tovar, 2015. Nutrition and atherosclerosis. Arch. Med. Res., 46: 408-426.
31. Wu, M.Y., C.J. Li, M.F. Hou and P.Y. Chu, 2017. New insights into the role of inflammation in the pathogenesis of atherosclerosis. Int. J. Mol. Sci., 10.3390/ijms18102034
32. Schwertani, A., H.Y. Choi and J. Genest, 2018. HDLs and the pathogenesis of atherosclerosis. Curr. Opin. Cardiol., 33: 311-316.
33. Prasad, K., 2012. Resveratrol, wine and atherosclerosis. Int. J. Angiol., 21: 7-18.
34. Voloshyna, I., S.M. Hussaini and A.B. Reiss, 2012. Resveratrol in cholesterol metabolism and atherosclerosis. J. Med. Food, 15: 763-773.